# Immunogenicity and safety of fractional doses of 17D-213 yellow fever vaccine in children (YEFE): a randomised, double-blind, non-inferiority substudy of a phase 4 trial

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## Summary

**Background** Current supply shortages constrain yellow fever vaccination activities, particularly outbreak response. Although fractional doses of all WHO-prequalified yellow fever vaccines have been shown to be safe and immunogenic in a randomised controlled trial in adults, they have not been evaluated in a randomised controlled trial in young children (9–59 months old). We aimed to assess the immunogenicity and safety of fractional doses compared with standard doses of the WHO-prequalified 17D-213 vaccine in young children.

**Methods** This substudy of the YEFE phase 4 study was conducted at the Epicentre Mbarara Research Centre (Mbarara, Uganda). Eligible children were aged 9–59 months without contraindications for vaccination, without history of previous yellow fever vaccination or infection and not requiring yellow fever vaccination for travelling. Participants were randomly assigned, using block randomisation, 1:1 to standard or fractional (one-fifth) dose of yellow fever vaccine. Investigators, participants, and laboratory personnel were blinded to group allocation. Participants were followed for immunogenicity and safety at 10 days, 28 days, and 1 year after vaccination. The primary outcome was non-inferiority in seroconversion (–10 percentage point margin) 28 days after vaccination measured by 50% plaque reduction neutralisation test ($PRNT_{50}$) in the per-protocol population. Safety and seroconversion at 10 days and 12–16 months after vaccination (given COVID-19 resctrictions) were secondary outcomes. This study is registered with ClinicalTrials.gov, NCT02991495.

**Findings** Between Feb 20, 2019, and Sept 9, 2019, 433 children were assessed, and 420 were randomly assigned to fractional dose (n=210) and to standard dose (n=210) 17D-213 vaccination. 28 days after vaccination, 202 (97%, 95% CI 95–99) of 207 participants in the fractional dose group and 191 (100%, 98–100) of 191 in the standard dose group seroconverted. The absolute difference in seroconversion between the study groups in the per-protocol population was –2 percentage points (95% CI –5 to 1). 154 (73%) of 210 participants in the fractional dose group and 168 (80%) of 210 in the standard dose group reported at least one adverse event 28 days after vaccination. At 10 days follow-up, seroconversion was lower in the fractional dose group than in the standard dose group. The most common adverse events were upper respiratory tract infections (n=221 [53%]), diarrhoea (n=68 [16%]), rhinorrhoea (n=49 [12%]), and conjunctivitis (n=28 [7%]). No difference was observed in incidence of adverse events and serious adverse events between study groups.

**Interpretation** Fractional doses of the 17D-213 vaccine were non-inferior to standard doses in inducing seroconversion 28 days after vaccination in children aged 9–59 months when assessed with $PRNT_{50}$, but we found fewer children seroconverted at 10 days. The results support consideration of the use of fractional dose of yellow fever vaccines in WHO recommendations for outbreak response in the event of a yellow fever vaccine shortage to include children.

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## Introduction

Yellow fever virus causes an acute viral haemorrhagic fever transmitted to humans by infected *Aedes* and *Haemagogus* mosquitos.[1] Yellow fever is endemic in 44 countries in tropical areas of Africa and Central and South America, where periodic outbreaks occur.[1] It has been estimated that, during outbreaks, around 12% of infections are severe, of which 47% result in death.[2] Vaccines to prevent yellow fever have been available since the 1930s; they are safe and effective and provide lifelong protection.[1] There are four WHO-prequalified yellow fever vaccines. These consist of a freeze-dried preparation of live attenuated yellow fever virus derived from the 17D strain (with vaccines derived from substrains 17DD and

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## Research in context

### Evidence before this study

We searched PubMed using the search term "(yellow fever vaccine) AND (fractional doses) AND (children)" for articles published between database inception and Feb 20, 2023, with no language restrictions. We identified no studies specifically assessing fractional dosing of yellow fever vaccines in children. An expert review and systematic review and meta-analysis highlighted the lack of information on the immunogenicity and safety of fractional doses of yellow fever vaccines in children.

### Added value of this study

To our knowledge, this is the first randomised trial assessing standard and fractional doses of yellow fever vaccines in children. The study provides information on the immunogenicity and safety of fractional doses of the yellow fever vaccine manufactured by the Chumakov Institute of Poliomyelitis and Viral Encephalitides at 10 days, 28 days, and 1 year after vaccination in children aged 9–59 months. The results show that fractional doses met the non-inferiority margin of –10 percentage points for seroconversion at 28 days after vaccination, and that high titres of neutralising antibodies were maintained at 1 year follow-up for a large percentage of vaccinees. At 10 days after vaccination, however, seroconversion and neutralising antibodies were significantly lower in the fractional dose group than in the standard dose group.

### Implications of all the available evidence

Our study supports the use of one-fifth fractional doses of yellow fever vaccines for children in the context of vaccine shortages in response to an outbreak and supports WHO policy on the use of fractional doses of yellow fever vaccine. Studies assessing the implications of a delayed immunological response in outbreak response and long-term protection are needed to better frame the use of fractional doses in response to outbreaks.

17D-204 and 17D-213, a substrain of the 17D-204). Vaccines are formulated to contain a minimum potency of 1000 IU per dose.[3] However, this minimum is not based on rigorous dose-finding studies in humans, and, in practice, vaccines contain potencies much higher than the minimum recommended.[4] Vaccines are administered subcutaneously or intramuscularly. Yellow fever vaccines are recommended for use in routine vaccination programmes in endemic countries, for travellers to endemic areas, and as part of outbreak response.[5] Yellow fever vaccination recommendations include children aged 9 months or older, and, in outbreak situations, when the risk of transmission may be very high, vaccination is considered for children aged 6 months or older. Owing to increased risk of serious adverse events, yellow fever vaccines are contraindicated for children younger than 6 months of age.[1]

In 2000, following a global shortage of vaccines, a stockpile of 2 million doses of yellow fever vaccines was reserved for outbreak response.[6] This was increased to 6 million doses in response to the global recognition of the yellow fever threat in 2003.[7] The outbreak response stockpile, however, has been insufficient to respond to large concurrent outbreaks, and fractional doses have been used as a dose-sparing strategy.[8–10] WHO recommendations on the use of fractional doses of yellow fever vaccines are based on limited data and, owing to lack of relevant information, exclude children younger than 2 years of age.[11,12] Fractional doses of yellow fever vaccine were studied in children aged 2 years or older following a large-scale campaign implemented in response to an outbreak in Kinshasa, the Democratic Republic of Congo.[8,9] This observational study showed seropositivity in 98% of children aged 24–59 months at 1 month and in 96% at 1 year.[8]

To broaden the evidence base for WHO recommendations, we designed a randomised controlled trial that included the evaluation of fractional doses in children 9–59 months of age.[13] This trial was preceded by the evaluation of fractional doses of all 4 WHO-prequalified vaccines in an adult population. The results of the adult study showed that fractional doses of all four WHO-prequalified vaccines were safe and immunogenic.[14] Following the evaluation of non-inferiority 28 days after vaccination and safety data in adults and considering supply and production capacity of the different manufacturers, and other planned studies assessing fractional doses of yellow fever vaccines, the study Data and Safety Monitoring Board (DSMB), as prespecified in our protocol, recommended one of the four WHO pre-qualified vaccines for evaluation of fractional doses in children. The DSMB recommended the 17D-213 yellow fever vaccine produced by the Federal State Unitary Enterprise of Chumakov Institute of Poliomyelitis and Viral Encephalitides for this study. Here, we present the results of a substudy of a fractional dose of this yellow fever vaccine compared with a standard dose in children aged 9–59 months of age.

## Methods

### Study design and participants

We conducted a double-blind, individually randomised substudy of the YEFE phase 4 trial in Mbarara, Uganda, to assess immunogenicity and safety of fractional doses of the 17D-213 yellow fever vaccine in children. The study took place at the Epicentre Mbarara Research Centre in Mbarara, Uganda. Mbarara district is located in the vicinity of Masaka and Rukungiri districts, which registered confirmed yellow fever cases in 2016.[15]

Participants were recruited from rural communities and through government health facilities. A group of social mobilisers, in liaison with health personnel and community leaders, conducted information sessions using locally adapted strategies. Parents or guardians of children interested in participating were invited to the study site. Written informed consent from the parent or guardian was required for the child to take part in the study.

Children were eligible to participate if they were 9–59 months of age, had no contraindications for yellow fever vaccination, had no history of previous yellow fever vaccination or infection, did not require yellow fever vaccination for travel during the course of the study, and were able to comply with study procedures. At screening, participants were tested for HIV using rapid diagnostic tests or DNA PCR test for children younger than 18 months following national guidelines.[16] Because the study included children of the age at which they would receive the combined measles and rubella vaccine, and to avoid immunological interference associated with the administration of two live-attenuated vaccines, a delay of 4 weeks was required between measles and rubella vaccination and yellow fever vaccination.[1] In practice, this meant that all participants had already received the measles and rubella vaccine at the time of receiving the yellow fever vaccine.

The study protocol was approved by the Research Ethics Review Committee of WHO (Geneva, Switzerland), the Scientific & Research and Ethics Committee of Mbarara University of Science and Technology Research Ethics Committee (Mbarara, Uganda), the Uganda National Council of Sciences and Technology (Kampala, Uganda), and the National Drug Authority (Kampala, Uganda). The trial was done in accordance with Good Clinical Practice guidelines.

### Randomisation and masking

Participants were randomly assigned, using block randomisation, 1:1 to standard or fractional (one-fifth) doses of yellow fever vaccine. A scratch-off booklet with unique allocation numbers was prepared by an independent statistician (DiagnoSearch LifeSciences, Mumbai, India) using a computer-generated random number list with non-disclosed variable block sizes of 6 or 8 within each age category. The booklets were stored securely and only used by the vaccination nurse once a participant was enrolled.

Vaccines were reconstituted and administered in the vaccination room, not accessible to other study staff. When preparing the allocated dose, the vaccination nurse covered the volume of the syringe with opaque tape to mask the dosage to participants and to the accompanying parent or guardian. The vaccination nurse and supervisor overseeing vaccination were aware of participant allocation arms but did not participate in further assessments and participant follow up visits. Personnel and investigators assessing outcomes were masked to dose throughout the entire duration of the trial.

### Procedures

In this study, we used one batch of standard 10-dose vials of the 17D-213 yellow fever vaccine manufactured by Chumakov Institute of Poliomyelitis and Viral Encephalitides (batch 598 released on Nov 13, 2017). This was chosen from the batches available at the time of the study with a potency closest to the internal minimum release specification. The potency of the vaccine was independently titrated at the National Institute for Biological Standards and Control (Potters Bar, UK) and contained 67 608 IU per dose (SD 1·15). The freeze-dried preparations and diluents were kept at 2–8°C at the central pharmacy and at the study site until administration. A vaccine vial was reconstituted once a participant had undergone all study-related procedures and was ready for vaccination. The reconstituted vial was kept in a vaccine carrier at 2–8°C per WHO and manufacturer's requirements. After 6 h of reconstitution, any remaining vaccine was discarded. Fractional dose consisted of one-fifth (0·1 mL) of the standard dose (0·5 mL). A single dose of the vaccine was administered subcutaneously in the deltoid region or in the upper outer lateral aspect of the thigh in children younger than 12 months of age or non-walking children using auto-disable syringes. Standard doses were administered using 0·5 mL auto-disable syringes at 45° injection angle, whereas fractional doses were administered using 0·1 mL auto-disable tuberculin syringes using a 90° injection angle to account for the shorter length of the needle.

At each study visit, participants had a medical consultation, and a 4 mL blood sample was collected at the initial visit before vaccination and at each follow-up visit. Follow-up visits were scheduled at 10 days (±1 day), 28 days (±3 days), and 365 days (±14 days) after vaccination. In practice, the final visit was done at 12 or 16 months (±14 days) after vaccination, because it was affected by control measures put in place to respond to the SARS-CoV-2 pandemic. Participants were also advised to return to the study site for a medical consultation at any time if there was a health concern.

Blood samples were processed and serum aliquoted into three aliquots at the study site laboratory within 4 h of blood collection. Serum samples were stored in –80°C freezers at the Epicentre Mbarara Research Centre laboratory until they were shipped to the Institut Pasteur Dakar (Dakar, Senegal) for virus neutralisation assay analysis and quantification. Neutralising antibody titres against yellow fever were assessed by 50% and 90% plaque reduction neutralisation tests ($PRNT_{50}$ and $PRNT_{90}$), as previously described.[17] Laboratory personnel were masked to study arm allocation.

During the medical consultations, adverse events occurring within the 28 days following vaccination were assessed. These included solicited and unsolicited events.

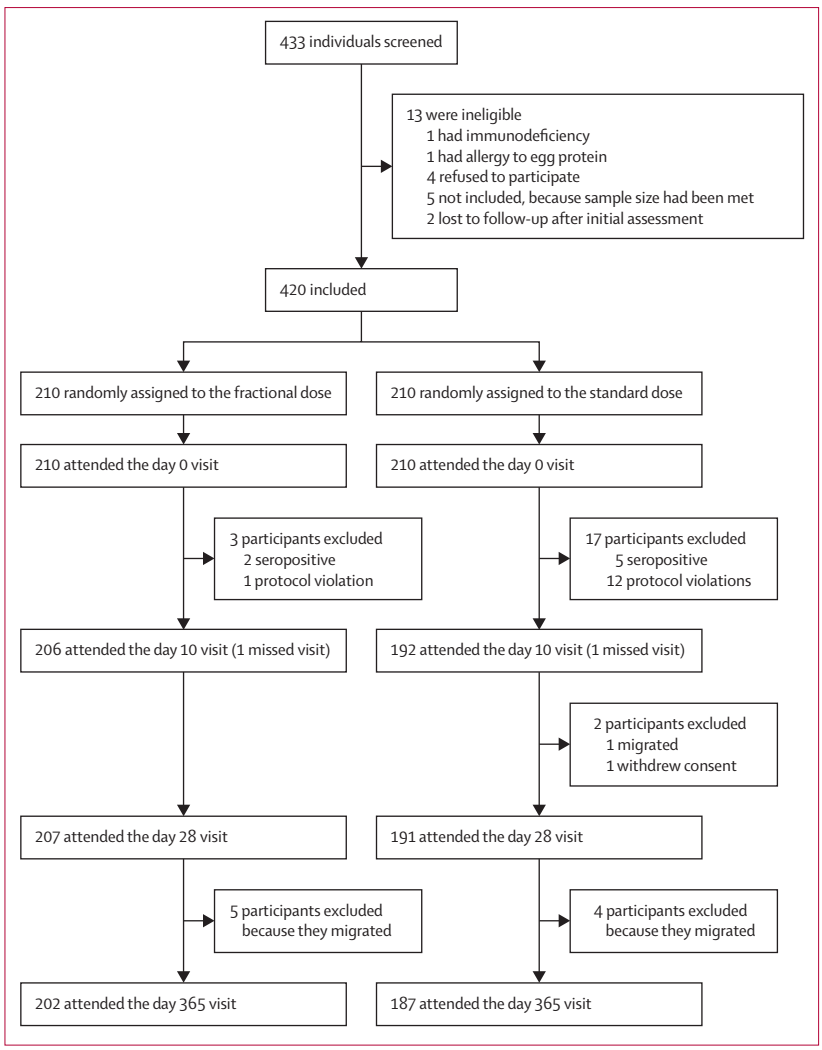


*Figure 1:* **Study profile**

All health-related problems were actively monitored and recorded 30 minutes after vaccination and at the 10-day and 28-day post-vaccination visits. During these visits, a clinician asked for the presence of local reaction, headache, fatigue, muscle pain, fever, gastrointestinal problem, and any other symptom since the previous visit. Parents or guardians were also asked to report any other symptoms or concerns during and outside scheduled visits. Serious adverse events, defined as any new health-related problem that occurred during follow-up that resulted in death, was life-threatening, necessitated hospital admission or prolongation of existing hospital stay, or resulted in disability or incapacity, were assessed throughout follow-up. All events were coded using the Medical Dictionary for Regulatory Activities, version 20.0. Adverse events were classified by the study investigators (doctors) as related to the study vaccine if they followed a temporal association with the yellow fever vaccination, had been previously associated with the vaccine, or there was no alternative plausible explanation.

### Outcomes

The primary outcome was non-inferiority in the proportion of participants in the per-protocol population seroconverting at 28 days after vaccination in the fractional dose group compared with the standard dose group. Non-inferiority was defined as no more than a 10 percentage point reduction in the proportion of seroconverted participants.

Secondary outcomes at day 28 after vaccination were assessment of geometric mean titres (GMT) and change in neutralising antibody titre from baseline (ie, geometric mean fold increase [GMFI]). Immunogenicity outcomes were also assessed at 10 days and 1 year after vaccination. Immunogenicity outcomes were assessed by $PRNT_{50}$ and $PRNT_{90}$. Safety and reactogenicity outcomes were the assessment of the occurrence of adverse events and serious adverse events during the 28 days after administration of the vaccine dose and serious adverse events throughout the duration of follow-up.

Seroconversion was defined as four-fold or greater increase in neutralising antibody titre compared with the pre-vaccination sample as measured by $PRNT_{50}$. For participants seronegative at baseline (ie, with a PRNT value below the limit of quantification [LOQ] <1:10), the baseline titre was converted to LOQ/2 and four-fold increase defined as a titre of 20. Any PRNT titre greater than 20 480 was designated 20 480, because this was the LOQ of titres. The fold increase was calculated as the ratio of the pre-vaccination titre compared with the post-vaccination titre at each visit. We did not test for cross-neutralisation with other flaviviruses.

### Statistical analysis

We assumed an overall 90% seroconversion in children 28 days after vaccination and considered that a minimum of 80% seroconversion should be achieved using fractional doses to ensure the protection level required to interrupt local transmission.[5,18] Considering a 2·5% significance level for a one-sided test, 90% power, and accounting for 5% loss to follow-up and 5% baseline yellow fever seropositivity, a sample size of 420 children was needed, with 210 participants per study group. To ensure representation of all age groups, quota sampling was used with an equal number (n=140) of children recruited from 9 months to 12 months, 13 months to 35 months, and 36 months to 59 months of age.

Analyses consisted of comparisons between the fractional dose group and standard dose group for children overall and by age category. The number and percentage of participants who seroconverted are presented by dose with two-sided exact Clopper-Pearson 95% CI. Non-inferiority was assessed by constructing a two-sided 95% CI using the Wilson score interval of the point difference between seroconversion percentages in

| | Overall | | 9–12 months old | | 13–35 months old | | 36–59 months old | |
|---|---|---|---|---|---|---|---|---|
| | Fractional dose group (n=210) | Standard dose group (n=210) | Fractional dose group (n=70) | Standard dose group (n=70) | Fractional dose group (n=70) | Standard dose group (n=70) | Fractional dose group (n=70) | Standard dose group (n=70) |
| Median age at enrolment (IQR), months | 24 (11–40) | 23 (11–42) | 11 (10–11) | 11 (10–11) | 24 (19–31) | 23 (18–30) | 46 (40–52) | 47 (42–53) |
| Sex | | | | | | | | |
| Female | 109 (52%) | 113 (54%) | 38 (54%) | 32 (46%) | 37 (53%) | 43 (61%) | 34 (49%) | 38 (54%) |
| Male | 101 (48%) | 97 (46%) | 32 (46%) | 38 (54%) | 33 (47%) | 27 (39%) | 36 (51%) | 32 (46%) |
| Moderate acute malnutrition* | 3 (1%) | 5 (2%) | 2 (3%) | 3 (4%) | 0 | 1 (1%) | 1 (1%) | 1 (1%) |
| Seropositive to yellow fever at baseline† | 2 (1%) | 5 (2%) | 0 | 0 | 2 (3%) | 2 (3%) | 2 (3%) | 1 (1%) |

Data are n (%) unless otherwise specified. *Mid-upper arm circumference ≤125 mm or weight-for-length/height z-score ≤–2. †Defined as 50% plaque reduction neutralisation test ≥10.

***Table 1:*** **Baseline characteristics**

the fractional and standard dose groups. Fractional doses were considered non-inferior if the lower bound of the CI for difference in seroconversion was greater than –10 percentage points. Two-sided 95% CIs of the mean difference between log GMT and log GMFI between the standard and fractional dose were generated using the *t*-distribution and then exponentiated to show the ratio of GMT and GMFI for the fractional dose compared with standard dose.

Immunogenicity outcomes were assessed in the per-protocol population and the intention-to-treat population. Analysis populations were defined for each timepoint. The per-protocol population included participants with a PRNT result at baseline and at the specific follow-up visit, who were seronegative ($PRNT_{50}$ <1:10) to yellow fever at baseline, and for whom the eligibility criteria were appropriately applied. The intention-to-treat population included any vaccinated participant with at least one $PRNT_{50}$ result after vaccination. Results presented are for the per-protocol population, with the results of the intention-to-treat population provided in the appendix. Adverse events and serious adverse events were summarised by study group and assessed in all vaccinated participants.

Data analysis was done in R, version 3.6.1. The DSMB regularly reviewed study data.

This study is registered with ClinicalTrials.gov, NCT02991495.

### Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

Between Feb 20 and Sept 9, 2019, 433 participants were assessed for eligibility, and 420 were enrolled; 210 were allocated to the standard dose and 210 allocated to the fractional dose (figure 1). An equal number of children were recruited from each age group (9–12 months, 13–35 months, and 36–59 months). Participants had a mean age of 27 months (SD 16), and 222 (53%) were female (table 1). 418 (>99%) of participants completed the 28-day post-vaccination visit, and 408 (97%) completed the last study visit. Owing to the SARS-CoV-2 pandemic, the 365 day follow-up visit for children was suspended between March 26 and July 26, 2020, and 192 children completed their last study follow-up visit at a mean of 480 days (SD 5)—ie, 16 months (±14 days) after vaccination. 124 (89%) of 140 children aged 9–12 months completed the last follow-up 12 months after vaccination, whereas 88 (63%) of 140 participants aged 13–35 and 94 (67%) of 140 aged 36–59 months completed their last follow-up visit 16 months after vaccination, with comparable distribution in the fractional and standard dose groups (appendix p 5). None of the children had HIV, and eight children met the definition of moderate acute malnutrition (table 1).

The most frequent reason for discontinuation from the study was migration out of the study area (n=11). One participant was discontinued from the study due to consent withdrawal. The primary per-protocol analysis at 28 days after vaccination included 398 (95%) participants.

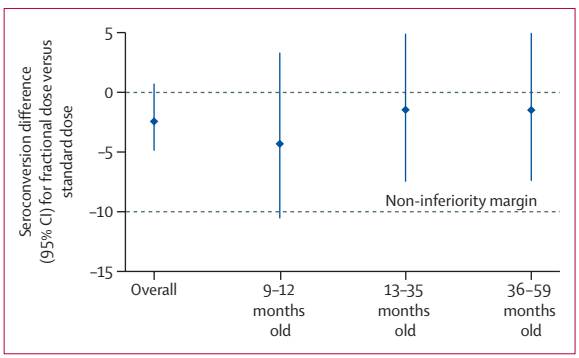


***Figure 2:*** **Non-inferiority of seroconversion of fractional dose compared with standard dose**

See Online for appendix

| | Seroconverted*, n/N (%, 95% CI) | Seroconversion difference†, percentage points (95% CI) | Geometric mean titre (95% CI) | Geometric mean titre‡ ratio (95% CI) |
|---|---|---|---|---|
| **Overall** | | | | |
| 10 days | .. | −18·56 (−27·45 to −10·11) | .. | 0·31 (0·21 to 0·47) |
| Fractional dose | 127/206 (61·7%, 54·6 to 68·3) | .. | 39 (29 to 51) | .. |
| Standard dose | 154/192 (80·2%, 73·9 to 85·6) | .. | 123 (91 to 167) | .. |
| 28 days | .. | −2·42 (−4·82 to 0·7) | .. | 0·82 (0·60 to 1·10) |
| Fractional dose | 202/207 (97·6%, 94·5 to 99·2) | .. | 1449 (1148 to 1828) | .. |
| Standard dose | 191/191 (100·0%, 98·1 to 100·0) | .. | 1774 (1465 to 2149) | .. |
| 12–16 months | .. | −4·63 (−9·7 to 0·54) | .. | 0·74 (0·52 to 1·06) |
| Fractional dose | 184/202 (91·1%, 86·3 to 94·6) | .. | 320 (249 to 412) | .. |
| Standard dose | 179/187 (95·7%, 91·7 to 98·1) | .. | 432 (333 to 561) | .. |
| **9–12 months old** | | | | |
| 10 days | .. | −1·26 (−17·2 to 14·54) | .. | 0·59 (0·31 to 1·11) |
| Fractional dose | 43/70 (61·4%, 49 to 72·8) | .. | 31 (21 to 45) | .. |
| Standard dose | 42/67 (62·7%, 50 to 74·2) | .. | 52 (31 to 86) | .. |
| 28 days | .. | −4·29 (−10·47 to 3·29) | .. | 0·86 (0·47 to 1·58) |
| Fractional dose | 67/70 (95·7%, 88 to 99·1) | .. | 1267 (774 to 2074) | .. |
| Standard dose | 66/66 (100%, 94·6 to 100·0) | .. | 1467 (1030 to 2090) | .. |
| 12–16 months | .. | −1·36 (−12·11 to 9·39) | .. | 0·78 (0·38 to 1·59) |
| Fractional dose | 60/67 (89·6%, 79·7 to 95·7) | .. | 398 (239 to 662) | .. |
| Standard dose | 60/66 (90·9%, 81·3 to 96·6) | .. | 508 (306 to 843) | .. |
| **13–35 months old** | | | | |
| 10 days | .. | −33·11 (−47·56 to −20·4) | .. | 0·18 (0·09 to 0·38) |
| Fractional dose | 40/68 (58·8%, 46·2 to 70·6) | .. | 53 (31 to 93) | .. |
| Standard dose | 57/62 (91·9%, 82·2 to 97·3) | .. | 289 (177 to 473) | .. |
| 28 days | .. | −1·45 (−7·4 to 4·86) | .. | 0·86 (0·55 to 1·33) |
| Fractional dose | 68/69 (98·6%, 92·2 to 100·0) | .. | 947 (682 to 1315) | .. |
| Standard dose | 62/62 (100%, 94·2 to 100·0) | .. | 1107 (821 to 1493) | .. |
| 12–16 months | .. | −13·23 (−23·08 to −2·72) | .. | 0·60 (0·31 to 1·18) |
| Fractional dose | 57/67 (85·1%, 74·3 to 92·6) | .. | 247 (151 to 405) | .. |
| Standard dose | 58/59 (98·3%, 90·9 to 100·0) | .. | 410 (256 to 654) | .. |
| **36–59 months old** | | | | |
| 10 days | .. | −22·60 (−37·23 to −9·24) | .. | 0·27 (0·13 to 0·54) |
| Fractional dose | 44/68 (64·7%, 52·2 to 75·9) | .. | 35 (22 to 56) | .. |
| Standard dose | 55/63 (87·3%, 76·5 to 94·4) | .. | 133 (78 to 225) | .. |
| 28 days | .. | −1·47 (−7·34 to 4·93) | .. | 0·74 (0·48 to 1·16) |
| Fractional dose | 67/68 (98·5%, 92·1 to 100·0) | .. | 2560 (1812 to 3616) | .. |
| Standard dose | 63/63 (100%, 94·3 to 100·0) | .. | 3446 (2595 to 4575) | .. |
| 12–16 months | .. | 0·14 (−6·94 to 6·68) | .. | 0·87 (0·54 to 1·41) |
| Fractional dose | 67/68 (98·5%, 92·1 to 100·0) | .. | 333 (248 to 447) | .. |
| Standard dose | 61/62 (98·4%, 91·3 to 100·0) | .. | 383 (261 to 562) | .. |

*Seroconversion is defined as ≥4-fold increase in neutralising antibody titre at each timepoint from baseline. †Seroconversion Difference=Fractional–Standard. ‡Geometric mean titre ratio=Fractional ÷ Standard.

***Table 2*: Seroconversion and geometric mean titre by $PRNT_{50}$ in the per-protocol population**

The main reasons for exclusion from the per-protocol analysis were protocol deviation related to a potential administration of an incomplete vaccine dose (n=12) and baseline seropositivity to yellow fever (n=7). There was one protocol violation that occurred in a participant that was vaccinated while febrile.

In the per-protocol population, 202 (98%, 95% CI 95 to 99) of 207 participants in the fractional dose group seroconverted by $PRNT_{50}$ at 28 days and 191 (100%, 95% CI 98 to 100) of 191 participants in the standard dose group. The difference in seroconversion between the fractional and the standard dose groups was –2 percentage points (95% CI –5 to 1; figure 2). The lower bound of the 95% CI for the difference in seroconversion between fractional and standard dose groups excluded the defined non-inferiority margin of –10 percentage

point, indicating non-inferiority of the fractional dose (table 2). Results for the intention-to-treat population were similar (appendix p 6). By $PRNT_{90}$, fractional doses did not reach non-inferiority compared with standard doses, with the lower bound of the 95% CI crossing the –10 percentage point non-inferiority margin (appendix p 6). Stratified by age group, smaller proportions of patients seroconverted with the fractional dose than with the standard dose, although these differences were not statistically significant (table 2).

At 10 days after vaccination, fewer patients in the fractional dose group seroconverted, with 127 (62%, 95% CI 55–68) of 206 children seroconverting by $PRNT_{50}$ compared with 154 (80%, 74–86) of 192 children in the standard dose group. Stratified by age group, standard doses were more immunogenic in children older than 12 months than in younger children aged 9–12 months, but responses to fractional doses were similar across age groups (table 2). At long-term follow-up (12 months or 16 months after vaccination), 184 (91%, 86–95) of 202 children in the fractional dose group seroconverted compared with 179 (96%, 92– 98) of 187 in the standard dose group. Seroconversion rates remained high at 16 months follow-up (appendix p 8).

At 28 days after vaccination the GMT of neutralising antibodies across all age groups was 1449 (95% CI 1148–1828) in the fractional dose group and 1774 (1465–2149) in the standard dose group (table 2). By age group, titres were higher in the 35–59 months age group in both groups compared with younger age groups (table 2). Trends were similar in the intention-to-treat population (appendix p 8). At 10 days after vaccination, GMTs were lower compared with 28 days and were significantly lower in the fractional dose group (39, 95% CI 29–51) compared with the standard dose group (123, 91–167). GMTs were lower in the fractional dose group than in the standard dose group for the three age groups at 10 days follow-up, but children aged 9–12 months also showed lower titres than other age groups in the standard dose group (table 2). At long-term follow-up, there was a substantial decrease in GMTs compared with 28 days follow-up, with mean titres decreasing from 1449 (95% CI 1148–1828) to 320 (249–412) in the fractional dose group and from 1774 (1465–2149) to 432 (333–561) in the standard dose group.

Owing to the small number of children with seropositivity to yellow fever at baseline, GMFI was nearly equal to GMT/5 (scalar based on the limit of detection). Hence, the comparison of fractional doses with standard doses using GMFI produced very similar results to the comparison of GMTs. GMFIs showed an increase in neutralising antibody titres from baseline at each timepoint for both study groups and reached the highest points at 28 days after vaccination (appendix p 13).

154 (73%) participants in the fractional dose group and 168 (80%) in the standard dose group reported at least one adverse event within the 28 days following vaccination. 11 children (5%) in the fractional dose group and 14 (7%) in the standard dose group reported an adverse event that was classified as related to the study vaccine. The most frequently reported adverse events were upper respiratory tract infections (n=221 [53%]), diarrhoea (n=68 [16%]), rhinorrhoea (n=49 [12%]), and conjunctivitis (n=28 [7%]). Most events were classified as mild (table 3; appendix 15,16). Four immediate adverse events (all pyrexia) occurred within 30 min following vaccine administration. Of these four, three were associated with pre-existing infections and infestations or general disorders and one was classified as related to the vaccine. 30 serious adverse events were reported among 26 (6%) participants during the study follow-up. These occurred between 6 days and 379 days after

| | Fractional dose group (n=210) | Standard dose group (n=210) |
|---|---|---|
| **Overall** | | |
| At least one adverse event | 154 (73%) | 168 (80%) |
| Vaccine-related adverse events | 11 (5%) | 14 (7%) |
| Severity | | |
| Mild | 152 (72%) | 162 (77%) |
| Moderate | 23 (11%) | 26 (12%) |
| Severe | 2 (1%) | 1 (<1%) |
| Life threatening | 0 | 0 |
| Serious adverse events | 12 (6%) | 18 (9%) |
| **By MedDRA system organ classes and preferred terms** | | |
| Infections and infestations | 10 (5%) | 13 (6%) |
| Bacteraemia | 1 (<1%) | 0 |
| Bronchiolitis | 1 (<1%) | 2 (1%) |
| Gastroenteritis | 1 (<1%) | 1 (<1%) |
| Perineal abscess | 0 | 1 (<1%) |
| Pneumonia | 3 (1%) | 7 (3%) |
| Sepsis | 2 (1%) | 0 |
| Subcutaneous abscess | 1 (<1%) | 1 (<1%) |
| Tonsilitis | 1 (<1%) | 0 |
| Urinary tract infection bacterial | 0 | 1 (<1%) |
| Injury, poisoning, and procedural complaints | 1 (<1%) | 1 (<1%) |
| Burns second degree | 0 | 1 (<1%) |
| Skin abrasion | 1 (<1%) | 0 |
| Metabolism and nutrition disorders | 0 | 2 (1%) |
| Dehydration | 0 | 2 (1%) |
| Nervous system disorders | 0 | 1 (<1%) |
| Infantile spasms | 0 | 1 (<1%) |
| Respiratory, thoracic and mediastinal disorders | 1 (<1%) | 1 (<1%) |
| Bronchial hyperreactivity | 0 | 1 (<1%) |
| Tonsillar hypertrophy | 1 (<1%) | 0 |

Data are n (%).

*Table 3:* **Adverse events up to 28 days after vaccination and serious adverse events throughout follow-up**

vaccination (mean 108 days, SD 106) and were mostly related to infections (table 3; appendix pp 19–20). All serious adverse events were classified as not related to the study vaccine.

## Discussion

We showed that fractional doses (one-fifth of the standard dose) of the 17D-213 yellow fever vaccine administered to children aged 9–59 months met the non-inferiority criterion for seroconversion by $PRNT_{50}$ 28 days after vaccination compared with the standard dose. At the long-term follow-up between 12 and 16 months after vaccination, most children continued to meet the definition of seroconversion (ie, ≥4-fold increase in neutralising antibody titre between the pre-immunisation and the 12-month or 16-month titre), although we detected a decrease in titres after the 28-day follow-up. GMTs were lower in the fractional dose group at all timepoints; however, the differences at 28 days and long-term follow-up were not statistically significant. There were no major safety concerns with any of the vaccine doses.

Our findings are similar those we have previously shown for adults, with high seroconversion at 28 days and 1 year.[14] However, compared with our results in adults, children had lower GMTs at 28 days and 12–16 months, and these were lower for the fractional dose compared with the standard dose. A study in the Democratic Republic of the Congo assessing fractional doses[8] also showed high seropositivity rates at 1 month and 1 year after vaccination, but, similar to our study, the youngest age group (children aged 24–59 months) had the lowest GMTs. Studies looking at long-term protection (8–10 years) of fractional doses in adults have shown encouraging results, with the short-term seroresponse being considered predictive of the long-term seroresponse.[19] However, the lower GMTs seen in children at 12–16 months after vaccination might indicate that protection may not be maintained. A systematic review and meta-analysis[20] estimated a seroprotection rate of 52% in children younger than 2 years more than 5 years after vaccination with standard doses, with a decay from rates close to 100% at up to 3 months after vaccination. Our study showed high seroconversion rates at 12–16 months after vaccination, but these were overall lower in the fractional dose group. Moreover, we detected lower titres in the fractional dose group compared with the standard dose group at 12–16 months after vaccination. Although these differences in seroconversion and GMTs were not significant, they could become more pronounced if the decay of antibodies is different in the two groups. Studies assessing the long-term duration of protection after administration of fractional doses are needed. Until more data are available on the long-term immunity, the use of fractional doses should be limited to outbreak response when there are insufficient standard doses and should not be considered for routine immunisation.

Although we showed non-inferiority of the fractional dose compared with standard dose 28 days after vaccination by $PRNT_{50}$, these results are not supported by the analysis by $PRNT_{90}$, with the lower bound of the 95% CI crossing the –10 percentage point non-inferiority margin. We consider that, as for other flaviviruses, results by $PRNT_{50}$ are preferred for the assessment of vaccine-induced immunity, because they provide more accurate results from the linear portion of the titration curve, with $PRNT_{90}$ titres considered more appropriate for epidemiological or diagnostic purposes.[21]

To our knowledge, this is the first assessment of yellow fever vaccine immunogenicity at 10 days after vaccination in children. Although this study was not powered to assess non-inferiority of the fractional doses at 10 days, we found statistically significant differences between fractional and standard doses, with 62% of children seroconverting in the fractional dose group and 80% in the standard dose group. Because fractional doses will be used in the context of outbreak response, this difference highlights the importance of early vaccination campaigns in outbreak response. However, the practical implications of a potential delayed protection in an outbreak response situation are not known. Additional studies, including more detailed time course studies and modelling, are warranted to better understand the early immune response to vaccination with fractional doses in children and could provide more insight into the practical implications of a delayed response in an outbreak.

This study has several limitations. First, the study is limited to the assessment of neutralising antibodies against yellow fever, and we did not assess for the presence of antibodies against other flaviviruses that could potentially interfere with the response to the yellow fever vaccine. Additionally, vaccination could induce protection through other antibody effector functions beyond neutralisation, with a likely role for T cell and memory B cell responses.[22] Understanding the vaccination induced immunity in children might bring a broader insight into interpretation of these results, because the immune response might be stronger and more persistent than shown here. Second, the identification of AEs relied on recall at 10 days after vaccination and could have resulted in an underreporting of events. However, study visits were the same in both arms, so the effect of recall bias is likely minimal. Additionally, the sample size was too small to detect rare serious adverse events associated with the vaccine.

Finally, the primary limitation for the generalisability of our findings is the vaccine used in this substudy. We used the 17D-213 yellow fever vaccine produced by Chumakov Institute of Poliomyelitis and Viral Encephalitides with potency closest to the manufacturer's minimum release specification. However, the vaccine had a potency 67 times higher than the minimum specification established by WHO, with one-fifth fractional doses exceeding the minimum by 13 times.

Ongoing studies assessing a low dose of 500 IU per dose compared with the standard dose of the 17D-204 vaccine in children aged 9–59 months Kenya and Uganda (NCT04059471)[13] and one-fifth and one-half doses compared with the standard dose of the 17DD vaccine in children aged 9–23 months in Uganda (NCT03725618) will provide further insight into the performance of lower doses in children and the applicability of fractional doses to other 17D vaccine substrains. Moreover, because they have a high risk of severe adverse reactions,[1] children younger than 9 months were excluded from the study. However, in outbreak responses, when the risk of yellow fever infection might be high, children aged 6 months or older might be offered vaccination.[1] The vaccination of children aged 6–8 months with fractional doses would need to be considered on a case-by-case basis, weighing risks and benefits.

Despite its limitations, the results of this study support the use of one-fifth fractional doses of yellow fever vaccines in children aged 9–59 months when there are insufficient standard doses to protect the at-risk population during an outbreak. The results of this study will widen the WHO policy on the use of fractional dosing of yellow fever vaccine to include children.

#### Contributors

AJ-G, KHG, AAS, DATC, PB, and RFG designed the study. MLN, DK, and GMW collected the data, KHG conducted the statistical analysis, and GF, MD, and NSB conducted the laboratory analyses. AJ-G and MLN prepared the first draft of the manuscript. AJ-G and RFG accessed and verified the data and vouch for the accuracy and completeness of the data and analyses reported. All authors contributed to the interpretation of data, critically reviewed the manuscript, and decided to publish the paper.

#### Declaration of interests

We declare no competing interests.

#### Data sharing

Data collected for the study, including deidentified participant data, data dictionary, and additional related documents, such as study protocol and statistical analysis plan, will be made available to others upon request to dpco@epicentre.msf.org, following Epicentre's data sharing policy and in accordance with WHO statement on public disclosure of clinical trial results.

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